Impairment of glutamate pathways by developmental exposure to environmental pollutants

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Mechanisms of Neurotoxicity and implications for neurological disorders

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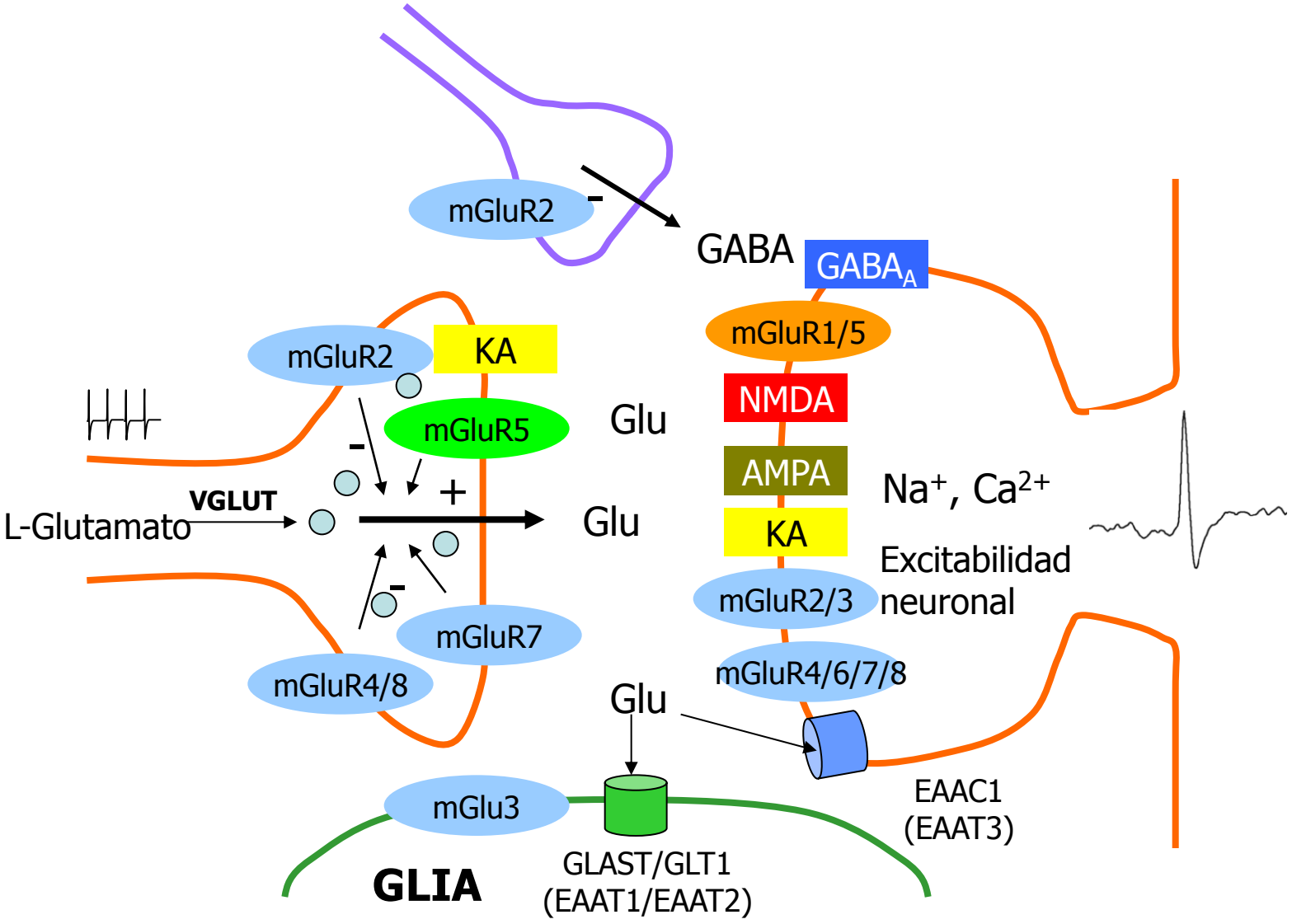
GLUTAMATE

- Non-essential amino acid
- Does not cross the BBB
- It serves as metabolic fuel, as neurotransmitter, as precursor of GABA neurotransmitter, as peptide-protein constituent
- Elevated brain concentration (≈ 14 000 nmol/mg)
- It is the most abundant excitatory neurotransmitter in the vertebrate nervous system: ≈ 80 - 90 % of neurons and synapses use glutamate
- It participates in cell differentiation, migration and survival in the developing brain
- It participates in learning (motor, sensorial, cognitive) processes
- It participates in memory formation
- Glutamate disfunction may “initiate / be part of” cell events leading to cell death

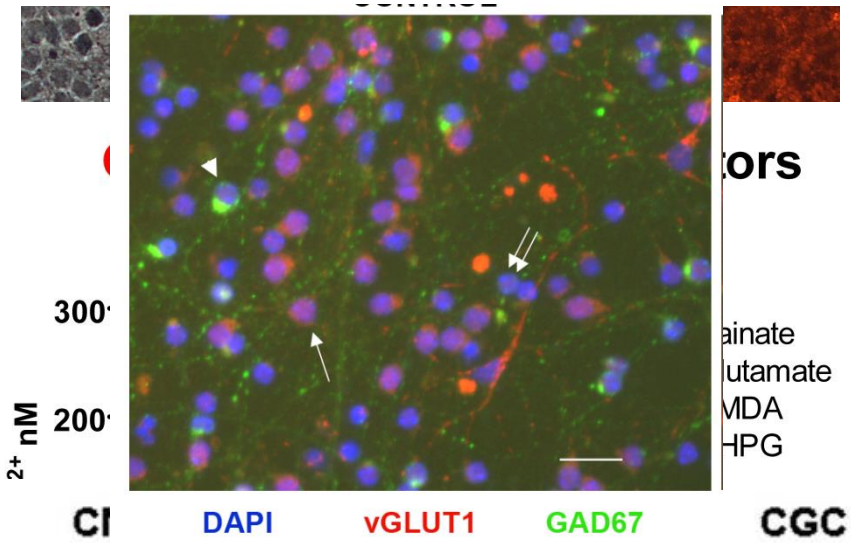
Glutamate-glutamate synapsis



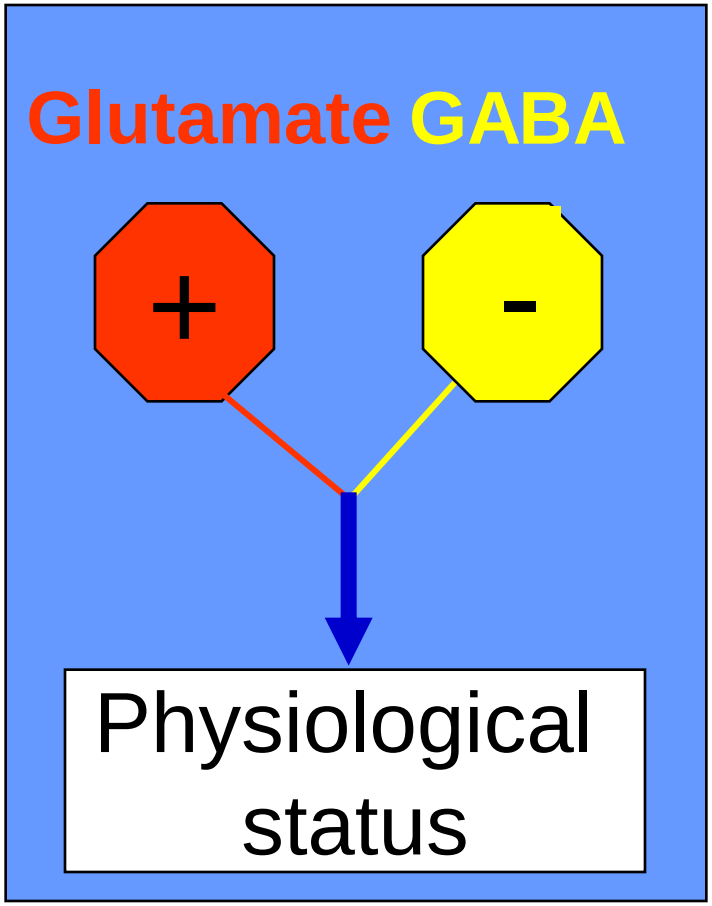
Artigas and Suñol, 2005

Experimental system: Primary neuronal cultures

- Cerebellar granule cells: constituted by glutamatergic neurons
- Cortical neurons: Glu, GABA and neurons
- Express glutamate receptors
- Express $GABA_A$ and glycine receptors
- Express ERs
- Express glutamate and GABA transporters



Brain control of neuronal excitability



Excess of glutamate neurotransmission
Defect of GABA neurotransmission

↓

EXCITOTOXICITY
CONVULSIONS

Excess of GABA neurotransmission

↓

CNS DEPRESSION

Priority List of Hazardous Substances (ATSDR) - 2011

The 50 top substances include:

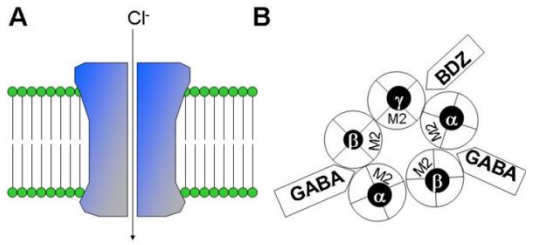
- 5 metals → Hg
- 7 PCB / Aroclor → PCB 138
- 3 DDTs → DDT
- 3 Hexachlorocyclohexane → Gamma-, beta- HCH
- 10 cyclodienes → Dieldrin, endosulfan
- 22 others

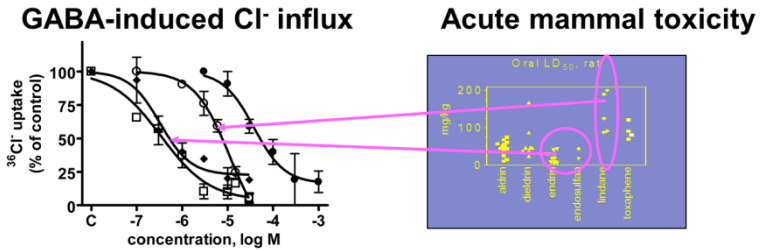
Short exposure to HCH and CD
Primary cultured neurons (30 min)

NO Effect on

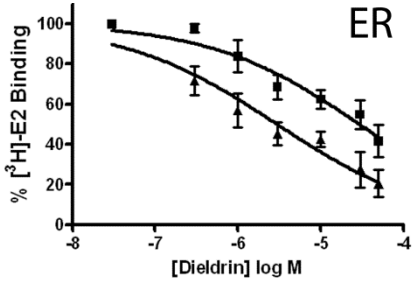
- glutamate uptake
- glutamate iR (NMDA, KA)

BUT, Effect on $GABA_A$ receptors





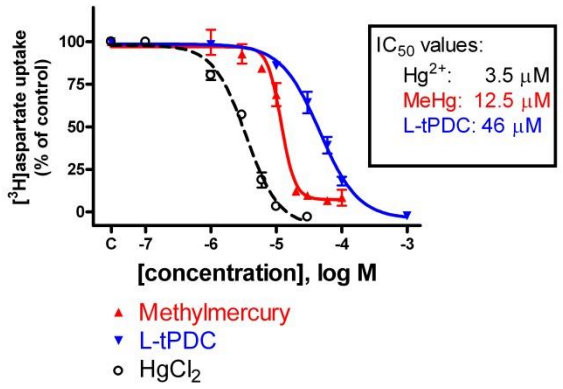
Effect on estrogen receptors



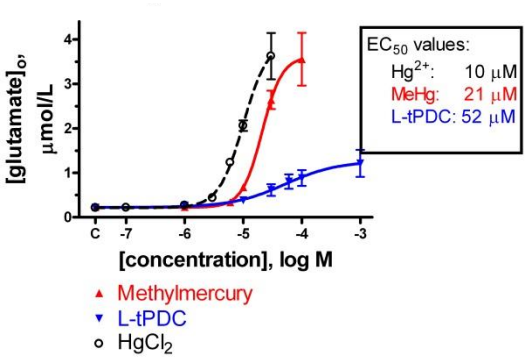
Vale et al., 2003; Babot et al., 2007;
Galofré et al., 2010; Briz et al., 2010, 2011

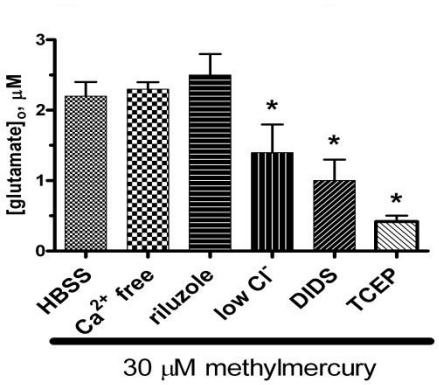
Short exposure to Hg
Cultured cerebellar granule cells (30 min)

Glutamate uptake

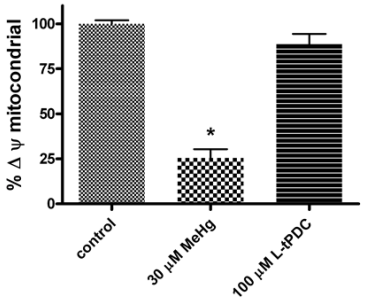


Net Glutamate release

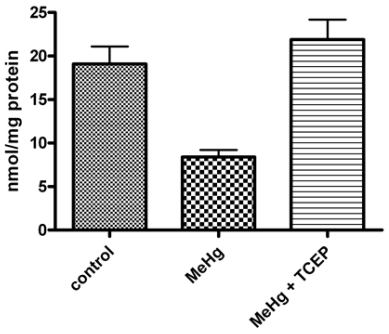




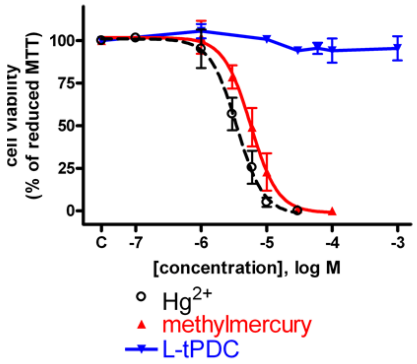
Mitochondria

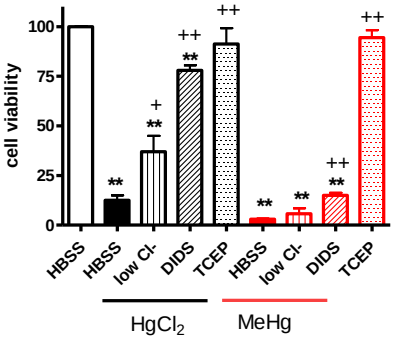


ATP



Cell death and neuroprotection





Fonfría et al., 2005, 2006

We worry about the long-term exposure to environmental toxicants in humans

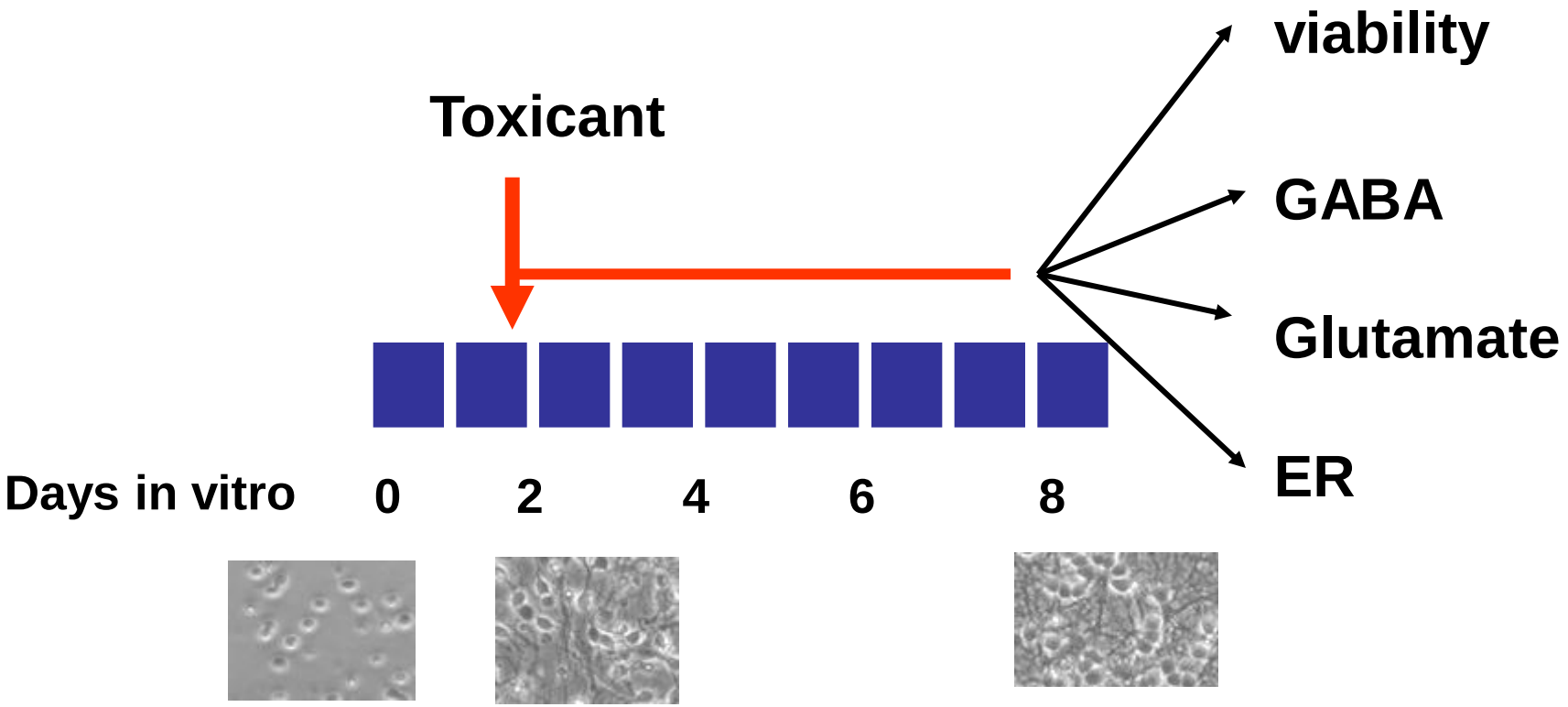
In nine healthy volunteers from New York, a minimum of 77 chemical contaminants were found in the same individual

Members of 13 families from 12 European countries, between 18 and 39 chemicals were found in any one individual

In 155 volunteers from 13 locations in the UK, up to 49 chemicals were found in any one person

Porta et al., 2012

Developmental toxicant exposure in cultured neurons

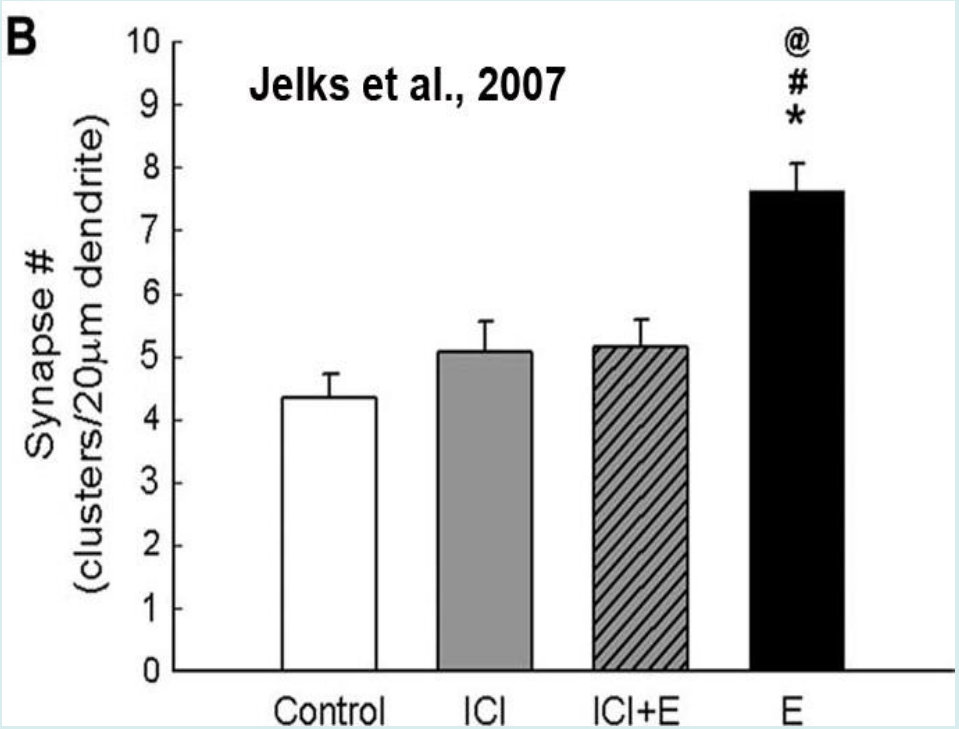


organochlorine pesticides in human samples

	Frequency (%) in:			
	placenta	Children Adipose tis	Adult Adipose tis	Adult serum
Σ DDT	99		≈100	
p,p'-DDE		79	39	> 50 %
Aldrin	26	12	40	
Dieldrin	23	8	29	
Endosulfan		14	78	> 50 %
Lindane (γ-HCH)	74	12	24	
β-HCH		>13	20	> 13%

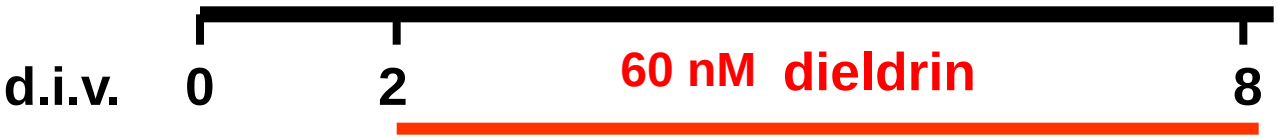
Botella et al., 2004; López-Espinosa et al., 2007; 2008
Molina et al., 2005; Porta et al., 2012; Younglai et al., 2002

The long-term effect of PCCAs – $GABA_AR$ blockers

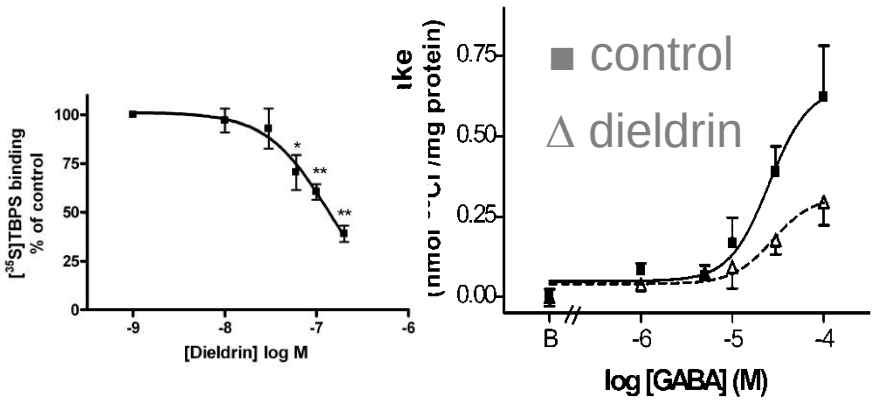


Long-term effect of dieldrin on the $GABA_A$ and estrogen receptor

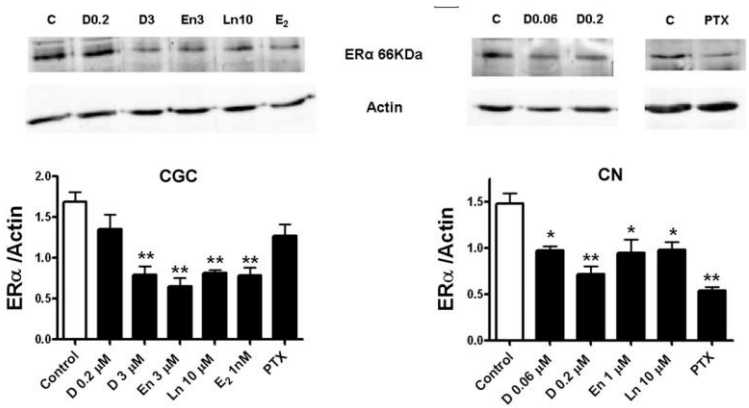
Primary neuronal cultured cells (cgc & ctx)



$GABA_AR$



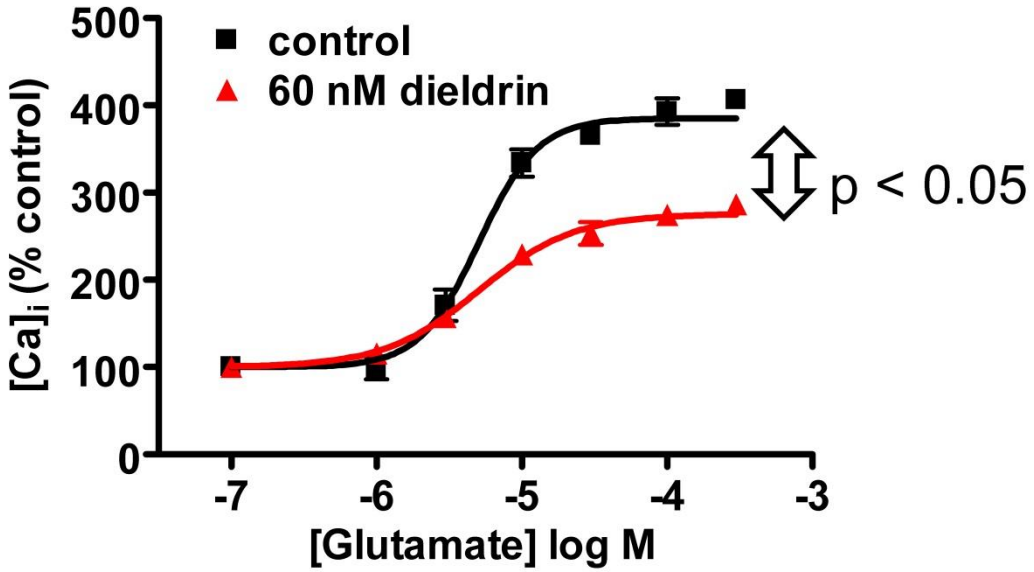
ER



Babot et al., Eur J Neurosci, 2007; Briz et al., Toxicol Sci, 2010; 2011

Prolonged exposure to dieldrin
Effect on glutamate receptors

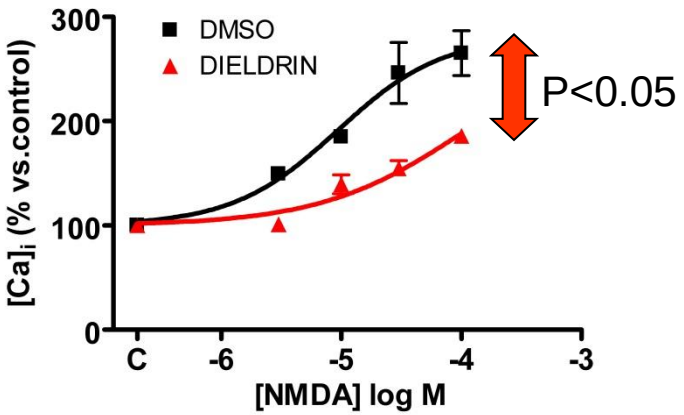
Cultured cortical neurons

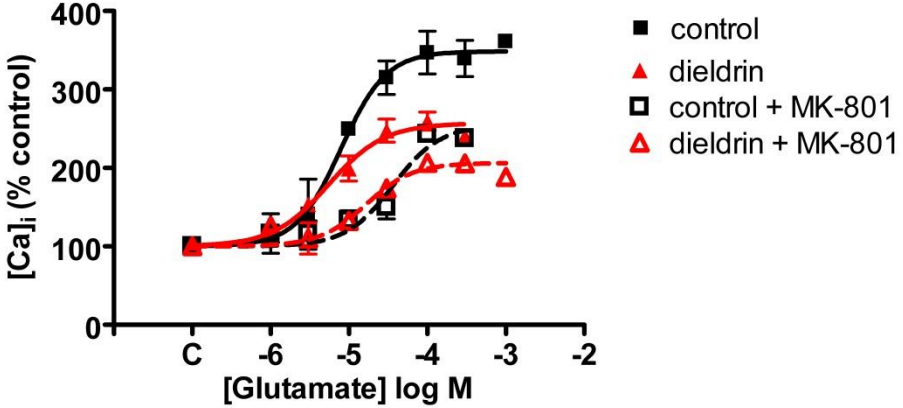


Briz et al., 2010

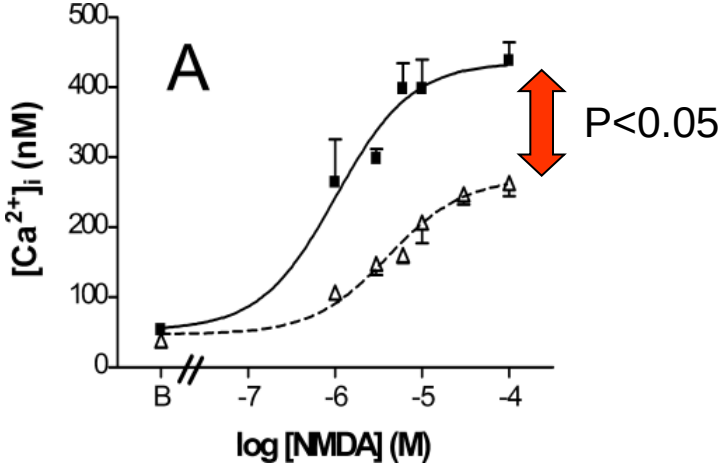
Effect on NMDA-glutamate receptor

Cortical neurons





Cerebellar granule neurons



Babot et al., 2007; Briz et al., 2010

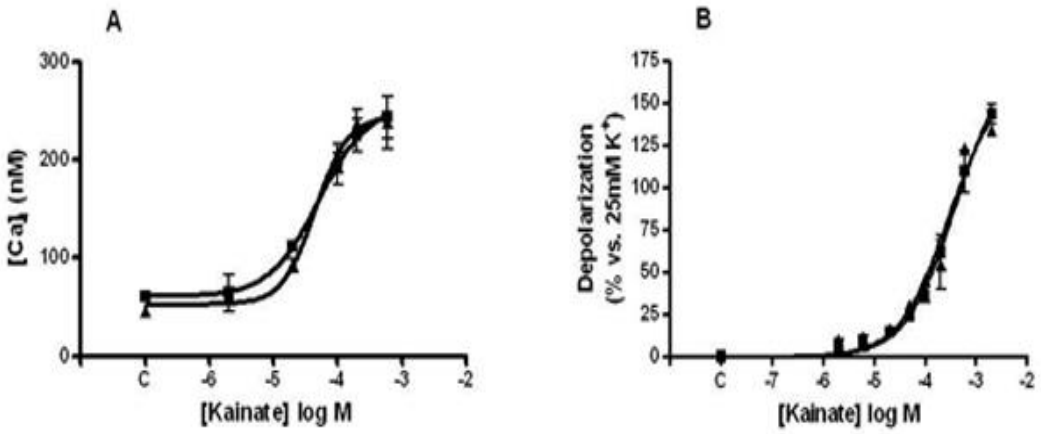
[3H]MK-801 binding

	Control	dieldrin
Kd (μM)	1.9±0.4	1.0±0.1
Bmax Pmol/mg prot	139±18	89±18*

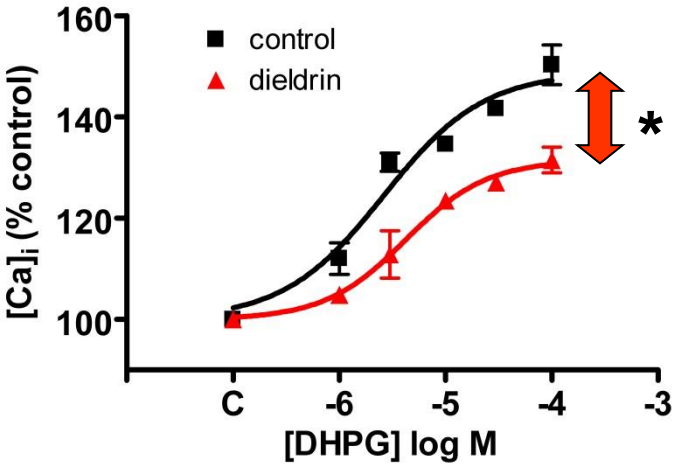
Other glutamate receptors are involved

Cortical neurons

AMPA/kainate receptor

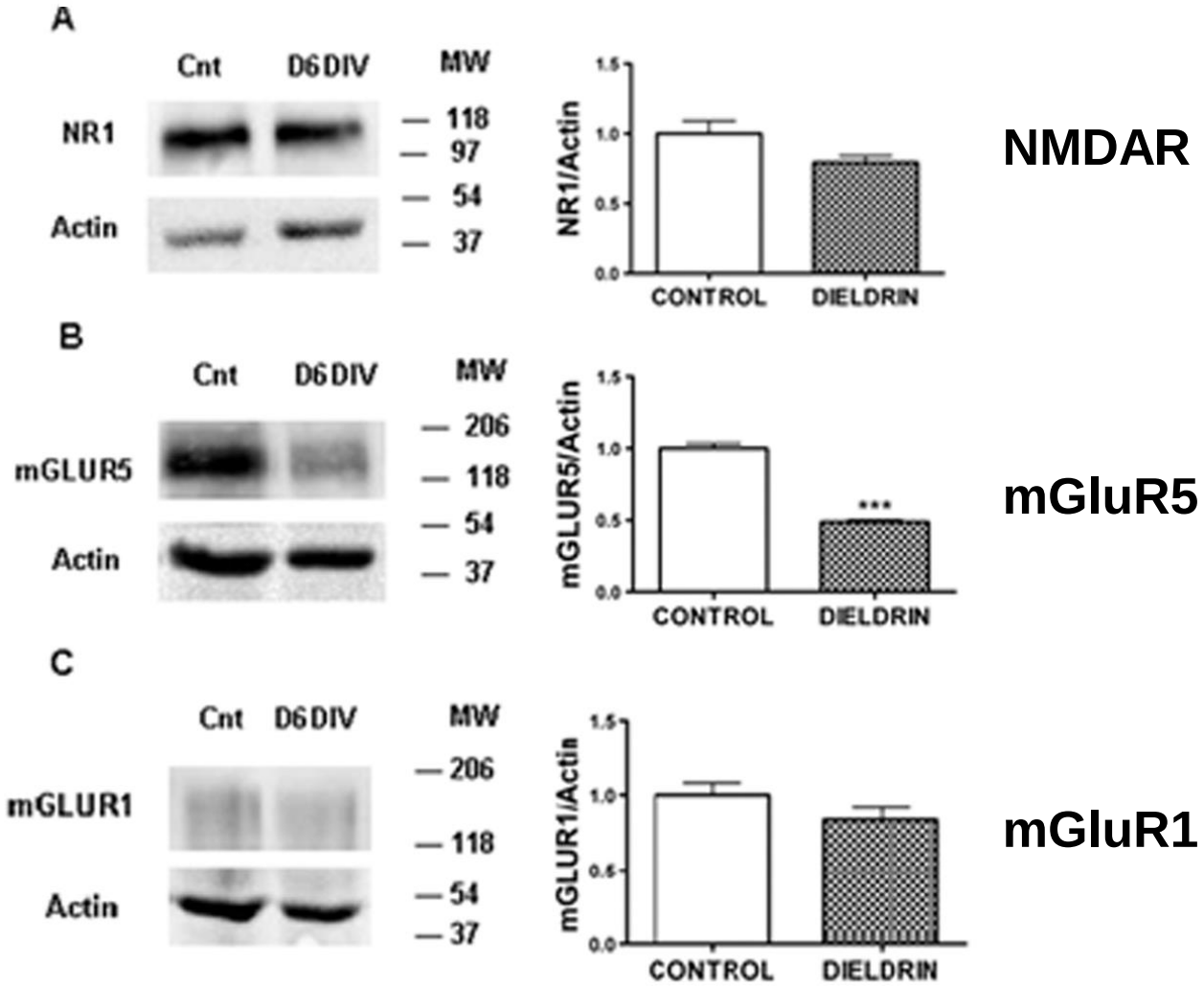


mGluR type I



Briz et al., 2010

Long-term exposure to dieldrin reduces mGluR5 protein levels, but not NMDAR NR1

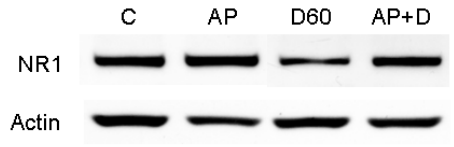


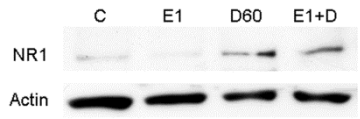
Briz et al., 2010

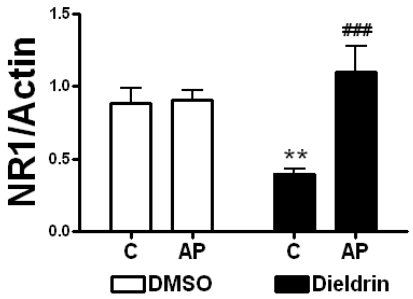
Long-term exposure to dieldrin vs NMDAR expression in the membrane and in the cytosol

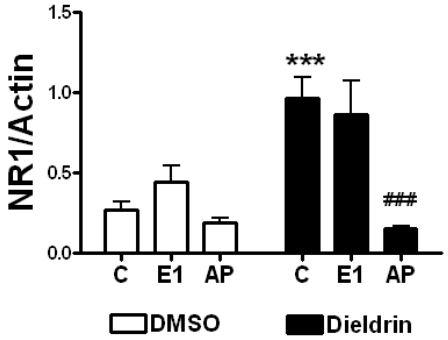
membrane **cytosol** **cell extract**

NR1 & NR2B

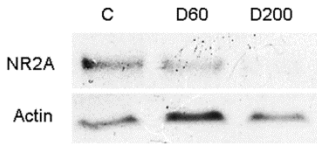


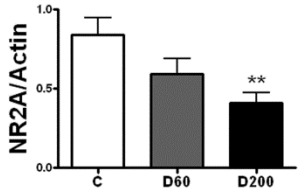


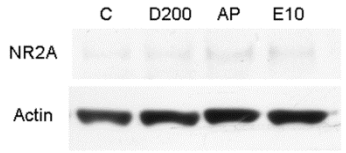


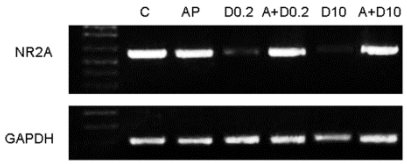


NR2A

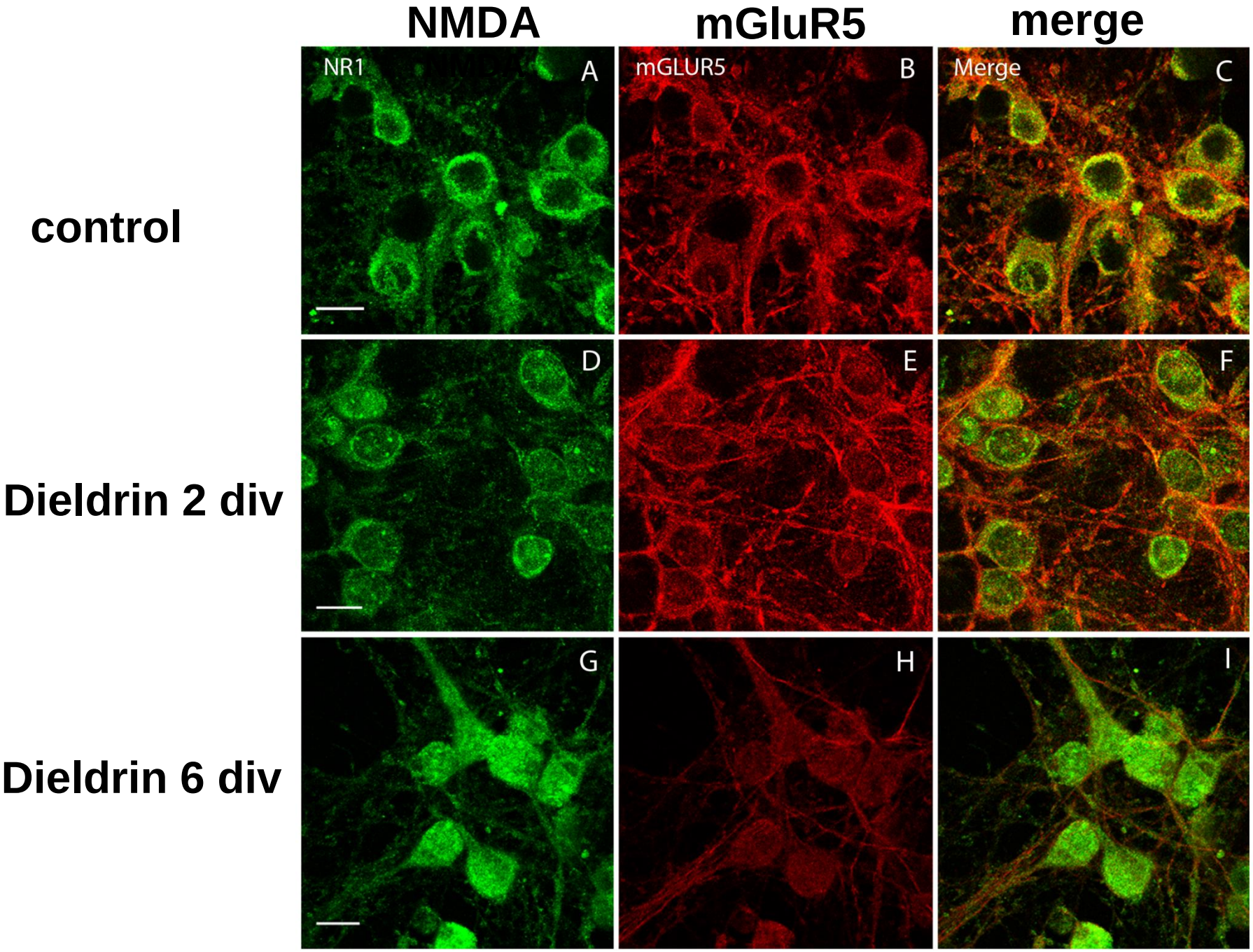








Briz et al., 2012



Briz et al., 2010

mercury in human samples

Was the cause of the Minamata disease in Japan and of the Iraq outbreak

Induces enduring neuropsychological deficits in children exposed during prenatal and early postnatal periods (Grandjean & Landrigan, Lancet 368, 2167-2178, 2006)

Hg blood cord levels in the range 0.1 - 135 µg/L from several newborn cohorts (Brazil, China, Japan, Spain, USA). US EPA RfD values for Hg: 5.8 µg/L (29 nM).

A high proportion of the individuals have Hg levels > RfD values

Targets for mercury neurotoxicity

- BBB
- Cytoskeleton
- Axonal transport
- Neurotransmission
- Cell signaling
- Protein synthesis, DNA, RNA
- Energy production
- Redox cell status

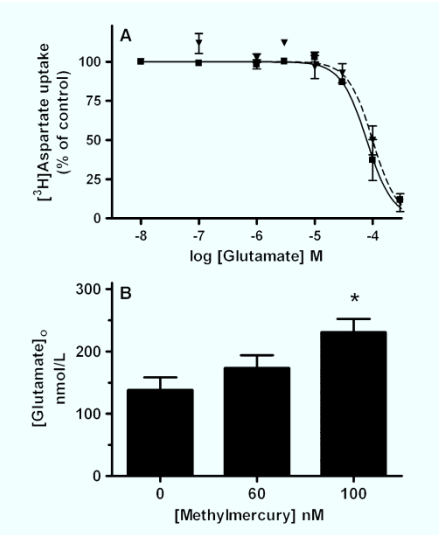
Methymercury reduces glutathion peroxidase (GPx) activity and increased extracellular glutamate in cerebellar granule cell

MeHg exposure: 100 – 300 nM for 4 – 7 days in vitro

CELL REDOX STATUS

	GSx	GSSG	GR	GPx	CAT	Glu uptake	$[Ca^{2+}]_i$
Control	93.84 ± 15.28	0.13 ± 0.03	52.66 ± 12.62	10.84 ± 0.65	0.34 ± 0.03	343.1 ± 69.8	132.1 ± 16.7
MeHg	102.4 ± 17.61	0.19 ± 0.03	52.61 ± 12.45	7.07 ± 0.44 **	0.40 ± 0.02	360.2 ± 57.7	148.9 ± 14.7

GLUTAMATE



Lipid peroxidation
Protein carbonylation
Cofilin and actin translocation to the mitochondria

ALL OF THEM PROTECTED BY THE ANTIOXIDANT PROBUCOL

Farina et al., Toxicol Sci 2009; Vendrell et al., 2007, 2010; Caballero et al., in preparation

Based on several assumptions:

- Glutamate is produced by the fetal liver and the excess of glutamate from the fetal circuit is removed by glutamate transporters in placenta
- Alterations of placental amino acid transporters and of cord blood amino acid levels have been related to various diseases and exposure to toxins

We determined:

- **Whether MeHg inhibits glutamate transport in human placenta**
- **Whether glutamate levels in cord blood are related to the exposure to Hg**

In collaboration with Dr Ferran Ballester, Valencian School of Health Studies and the Department of Public Health, Univ Miguel Hernández, and CIBERESP

The mother-child INMA cohorts

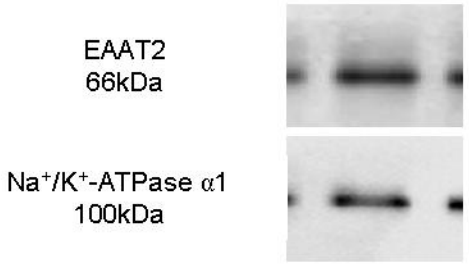
INMA - Childhood and Environment *is a research network of several Spanish groups that created a project with the aim to study the paper of the more relevant environmental pollutants in the air, water and diet during the pregnancy and beginning of life, and their effects in the growth and development.*

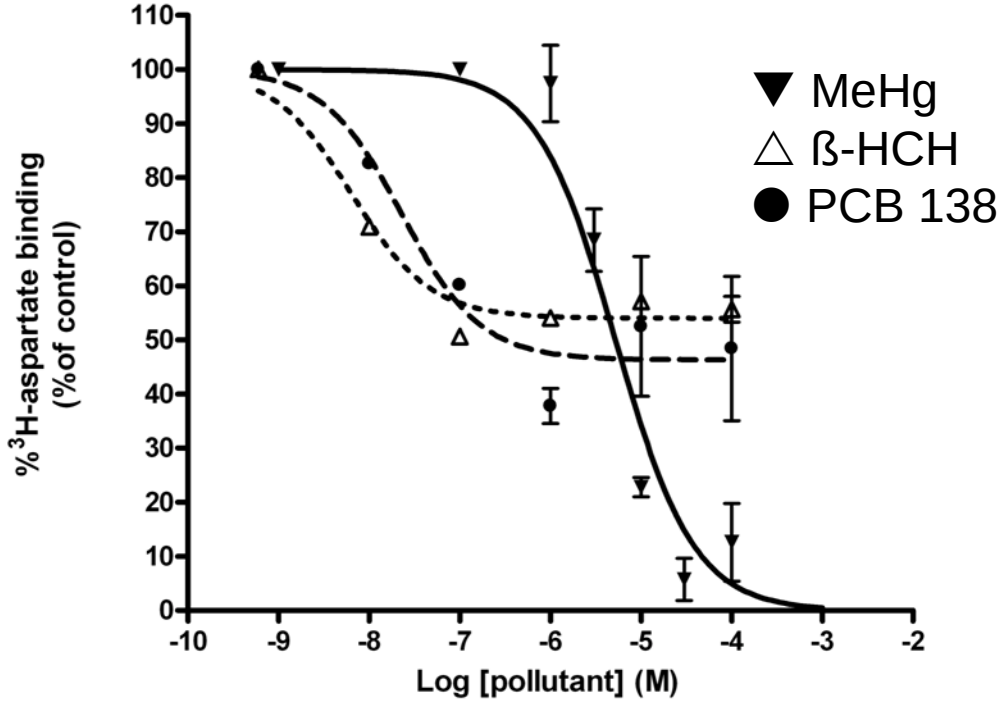
~ 1500 mother–child pairs with complete information on maternal / cord blood levels of organochlorine compounds and Hg and child neuropsychological assessment (Bayley Scales of Infant Development) at age 14 months.

- prenatal PCB exposure, particularly to congeners 138 and 153, resulted in impairment of psychomotor development a negative association between prenatal exposure
- to total mercury and psychomotor development among female infants

Forns et al., Environ Int 45, 72, 2012; Llop et al., Amer J Epidemiol 175, 451, 2012

Inhibition of glutamate transport in human placenta





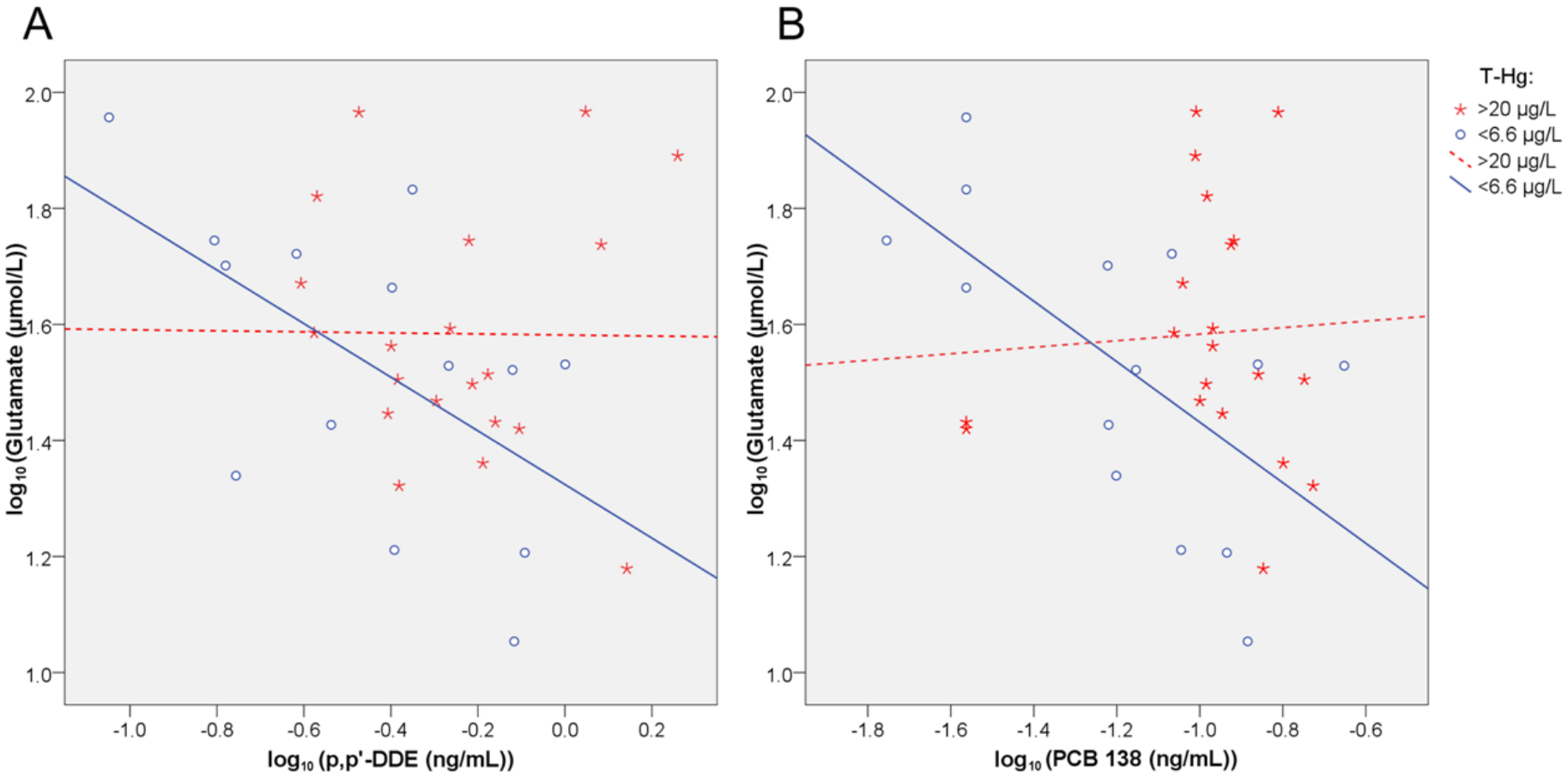
Palou et al., in preparation

Association between glutamate and pollutant levels in cord blood

40 cord blood samples from the INMA cohort of Valencia, Spain
Hg levels from non detectable to 66 ng/ml (mean value: 9,2 ng/mL)

		Crude			Lipid adjusted			Lipid & age adjusted		
	n	coef[c]	95% CI	p-value	coef[c]	95% CI	p-value	coef[c]	95% CI	p-value
log_{10} T-Hg	34	8.2	(-17.8; 42.5)	0.564	3.9	(-20.0; 35.0)	0.766	23.7	(-10.3; 70.8)	0.187
T-Hg<6.6	14	Reference			reference			reference		
T-Hg>20	20	12.9	(-23.1; 65.7)	0.525	6.1	(-26.4; 52.9)	0.743	33.0	(-14.4; 107)	0.197
log_{10}HCB	34	-28.4	(-51.5; 5.7)	0.090	-29.0	(-50.5; 1.8)	0.062	-28.0	(-54.4; 13.6)	0.152
log_{10}bHCH	34	-17.6	(-37.3; 8.2)	0.157	-23.6	(-40.5; -1.8)	0.036	-23.3	(-43.6; 4.3)	0.088
log_{10}4,4'-DDE	34	-35.6	(-66.2; 22.8)	0.174	-41.3	(-67.6; 6.5)	0.078	-37.0	(-67.5; 21.9)	0.163
4,4'-DDT<LOD	18	Reference			reference			reference		
4,4'-DDT>LOD	16	-25.6	(-48.4; 7.2)	0.109	-15.6	(-42.1; 23.0)	0.365	-13.3	(-40.7; 26.9)	0.451
log_{10}PCB118	34	1.6	(-38.7; 68.3)	0.951	0.0	(-37.7; 60.6)	0.998	2.2	(-36.3; 63.9)	0.926
log_{10}PCB138	34	-38.9	(-68.6; 18.9)	0.141	-51.1	(-73.5; -9.9)	0.023	-48.5	(-72.7; -2.7)	0.041
log_{10}PCB153	34	-26.7	(-57.9; 27.8)	0.264	-32.7	(-59.8; 12.6)	0.127	-27.7	(-59.3; 28.4)	0.258
log_{10}PCB180	34	-41.2	(-71.6; 21.8)	0.147	-47.9	(-73.3; 1.9)	0.056	-44.5	(-72.3; 11.4)	0.095
$log_{10}(\Sigma_4$PCBs)	34	-42.3	(-73.3; 24.3)	0.154	-48.4	(-74.6; 4.7)	0.066	-44.3	(-73.8; 18.3)	0.123
$log_{10}(\Sigma_8$OCs)	34	-48.4	(-75.3; 7.6)	0.076	-54.8	(-76.9; -11.7)	0.022	-55.1	(-80.0; 0.8)	0.052

Correlation in samples with low / high Hg levels



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